

## RESULTS

### MATURITY STAGES OF SAND GOBY (*Oxyeleotris marmoratus*) OVARY

Gross examination of the paired ovaries revealed that the stage of ovarian development could be classified into immature, mature and gravid stages. In the immature stage, ovaries were small, thread-like, pale yellow-orange in color, occupied one-third of the ventral cavity and the average GSI was  $0.28 \pm 0.14$ . In the mature stage, ovaries were orange in color, 1.5–4 mm in diameter, occupied one-half of ventral cavity and the average GSI was  $0.81 \pm 0.44$ . In the gravid stage, ovaries were swollen, yellow in color, 2.5–6.5 mm in diameter, fully occupied ventral cavity, and the average GSI was  $2.75 \pm 1.51$ . All stages were found throughout the year, except in November, only the gravid stage was identified.

### GENERAL MORPHOLOGY OF SAND GOBY (*Oxyeleotris marmoratus*) PITUITARY GLAND

The sand goby pituitary gland consisted of the adenohypophysis and the neurohypophysis. The adenohypophysis was divided into three regions: the rostral pars distalis (RPD), the proximal pars distalis (PPD) and the pars intermedia (PI). The rostral pars distalis was separated from the rest of the pituitary gland by a distinct circumferential constriction. (Fig 1A, B and C). Pituitary glands were divided into 3 groups according to the said maturity stages of ovary. The size of PPD increased in the mature stage and reached maximum size at the gravid stage (Fig 1B and C).

### CELLULAR COMPOSITION OF SAND GOBY (*Oxyeleotris marmoratus*) PITUITARY GLAND

Sagittal sections of the sand goby pituitary gland stained with Masson's Trichrome showed the various cell types, segregated into three zones of the adenohypophysis. In the RPD, acidophils formed the major component, whereas the PPD consisted mainly of two cell types: basophils and acidophils (Fig 2A, 3A and 4A). Basophils appeared to be homologous with the somatotroph and gonadotroph described in other teleosts. In the gravid stage, basophils showed a marked numerical increase, whereas in the immature and mature stages, the basophils were comparatively few and a great number of vacuoles was observed in areas of the PPD normally occupied by the basophils. (Fig 2B, 3B and 4B). The neurohypophysial

processes originating in hypothalamic regions interdigitate with all three zones through the pituitary stalk. The neurohypophysis, establishing the pars nervosa (PN), was especially deep and elaborate with the PI.

## IMMUNOHISTOCHEMISTRY

The anti-GTH II  $\beta$  (LH) stained most of basophils and the optimal dilution of GTH I  $\beta$  (LH) antiserum is 1:8000. The strong anti-GTH II  $\beta$  (LH) labeling gonadotrophs were found in the PPD of the sand goby pituitary gland in all stages (Fig 5A, 6A and 7A). In the gravid stage, the anti-GTH II  $\beta$ (LH) labeling gonadotrophs showed a marked numerical increase whereas in the immature and mature stages, the anti-GTH II  $\beta$  (LH) labeling gonadotrophs were comparatively few and a great number of vacuoles was observed in areas of the PPD normally occupied by the gonadotrophs (Fig 5B, 6B and 7B). In the immature and mature stages, the GTH II  $\beta$  (LH) immunoreactivity is found inside in some vacuoles (Fig 5B and 6B). In the gravid stage, the number of vacuoles was marked numerical reduces because most of the vacuoles are fully filled with the LH hormone (Fig 7B). The number of anti- GTH II  $\beta$  (LH) labeling gonadotrophs in the mature stage ( $35.5 \pm 4.4$  cell/mm<sup>2</sup>) was significantly higher than that of the immature stage ( $48.3 \pm 7.2$  cell/mm<sup>2</sup>) ( $P < 0.05$ ). In addition, the number of anti-GTH II  $\beta$  (LH) labeling gonadotropes in the gravid stage ( $60.1 \pm 3.5$  cell/mm<sup>2</sup>) was significantly higher than that of the immature ( $P < 0.05$ ) and mature stages ( $P < 0.05$ ) (Fig 8). Interestingly, anti-GTH I  $\beta$  (FSH) labeling gonadotrophs were not found in any stage in the various dilutions of anti-GTH I  $\beta$  (FSH) antiserum. No immunoreactivity was found in the negative control section.

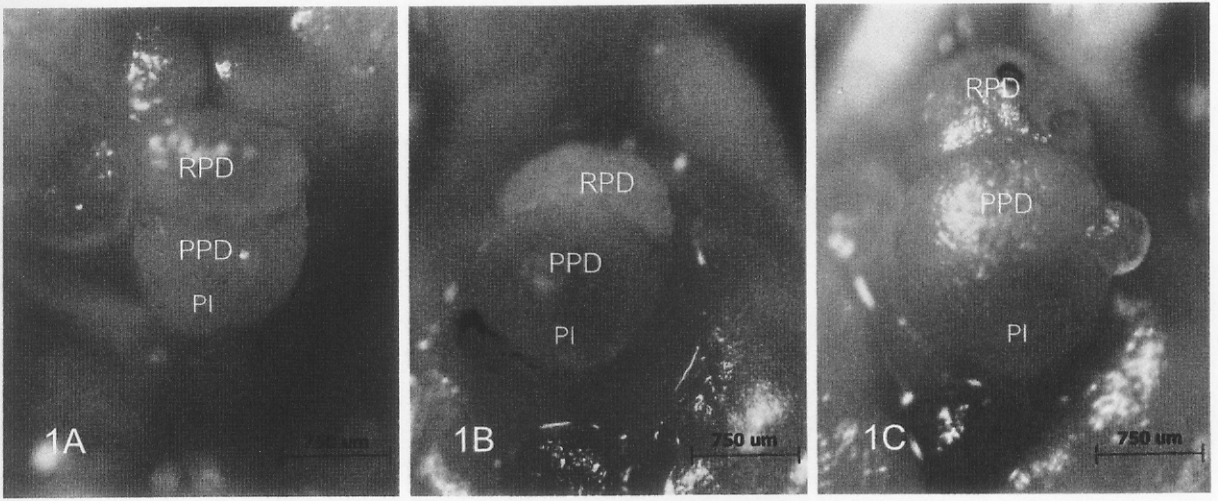
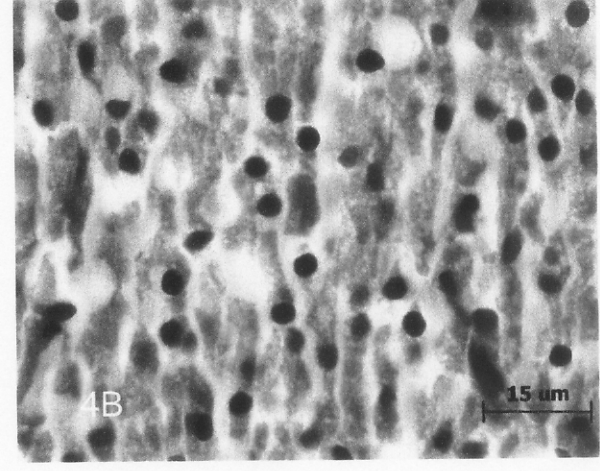
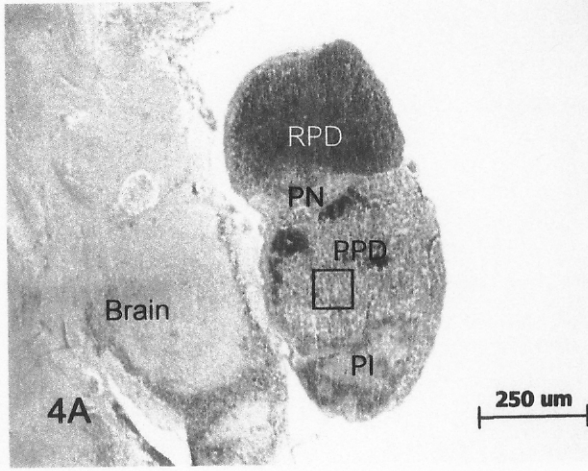
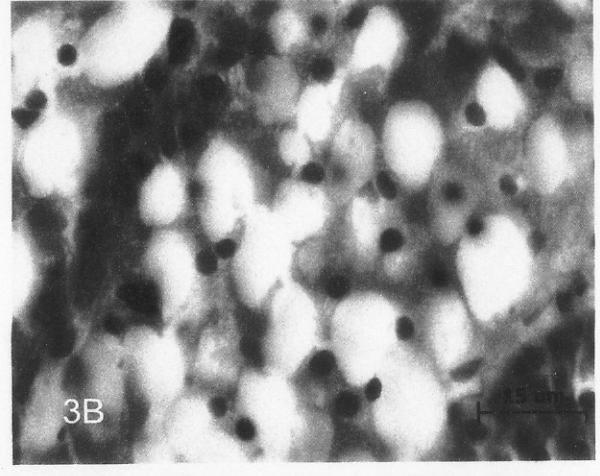
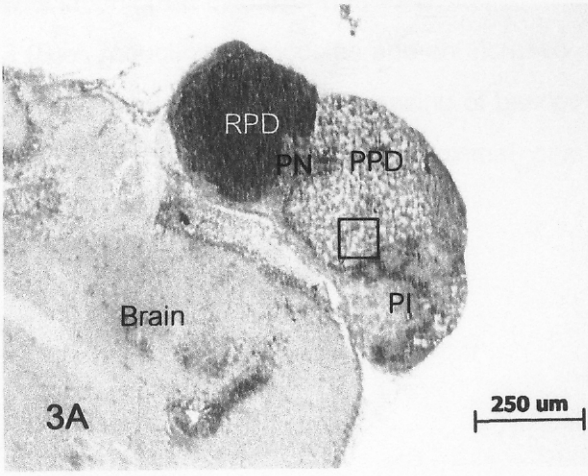
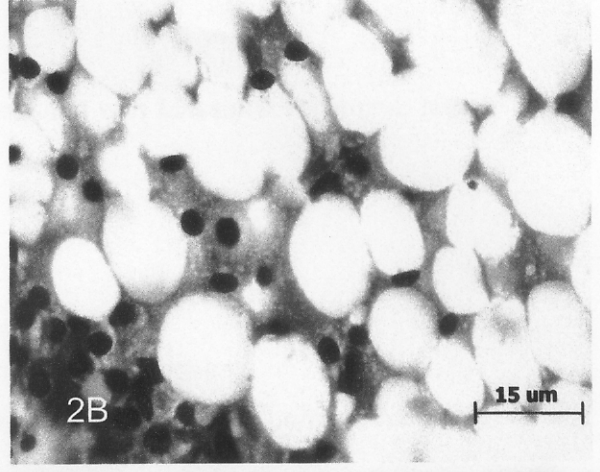
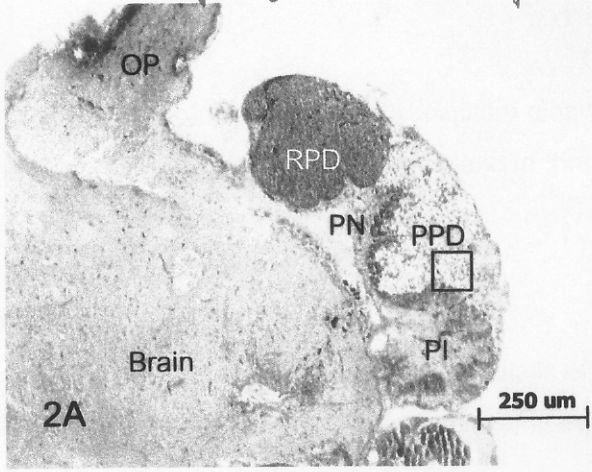


Fig 1. General morphology of the sand gobies (*Oxyeleotris marmoratus*) pituitary gland at different maturity stages of the ovary: (A) immature, (B) mature, and (C) gravid stage.

RPD, Rostral pars distalis; PPD, Proximal pars distalis; PI, Pars intermedia.

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Sagittal sections of the sand goby's pituitary gland, stained with Masson's Trichrome, shows the cellular composition of the pituitary gland in three stages:

Fig 2 (A) Immature stage

Fig 3 (A) Mature stage

Fig 4 (A) Gravid stage

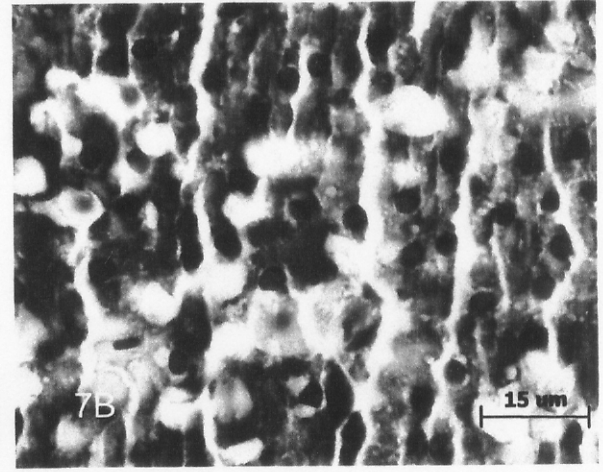
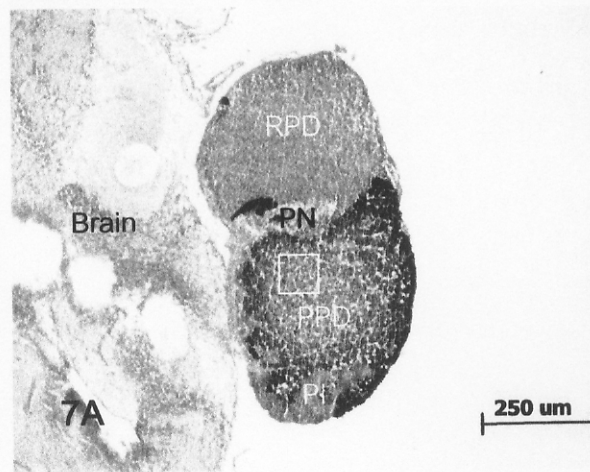
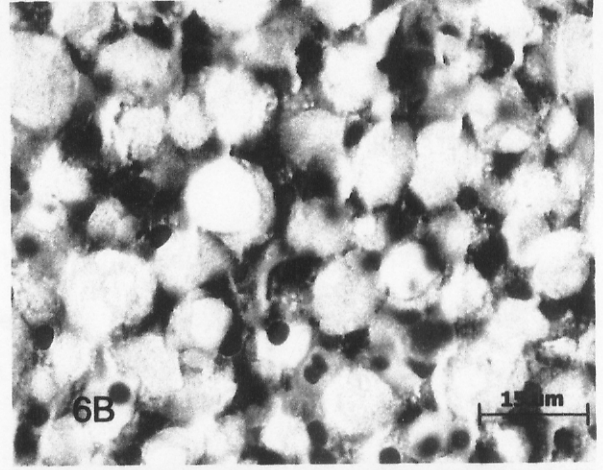
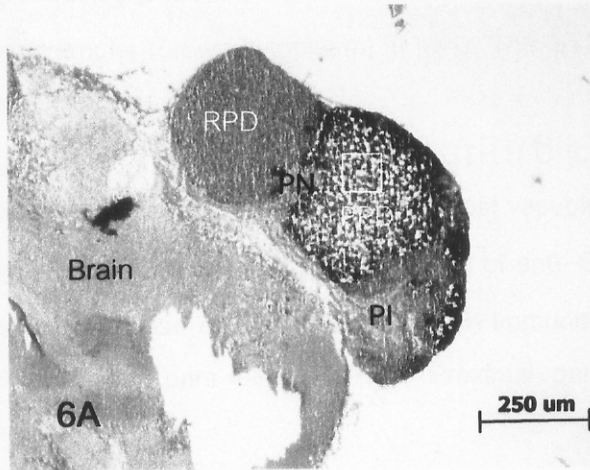
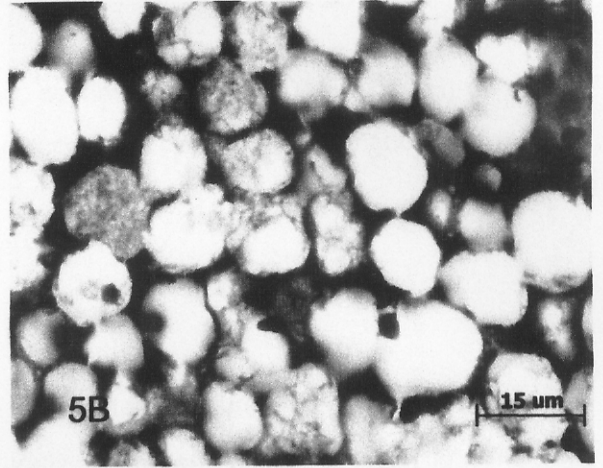
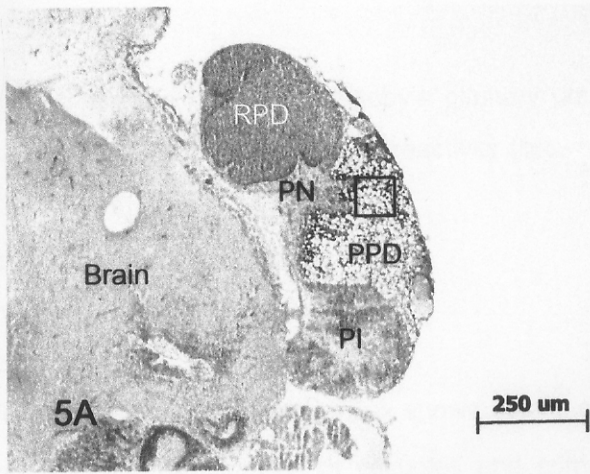
In each of these, the enlarged box shows details of the cellular structure:

Fig 2(B) A great number of vacuoles and comparatively few basophils (blue cytoplasm), and a few acidophil (red cytoplasm) in PPD.

Fig 3 (B) A reduction of vacuoles and an increase of basophils in PPD.

Fig 4 (B) A marked numerical increasing of basophils in PPD.

RPD, Rostral pars distalis; PPD, Proximal pars distalis; PI, Pars intermedia; PN, Pars nervosa; OP, Optic nerve.



Sagittal sections of the sand goby's pituitary gland, stained with anti- GTH II  $\beta$  (LH) at a dilution of 1: 8000, shows immunoreactivity (brown color) in the cytoplasm of gonadotrophs in PPD in three stages:

Fig 5 (A) Immature stage

Fig 6 (A) Mature stage

Fig 7 (A) Gravid stage

In each of these, the enlarged box shows details of the cellular structure:

Fig 5 (B) A great number of vacuoles and comparatively few anti-GTH II  $\beta$  (LH) labeling gonadotrophs (brown cytoplasm) in PPD. The GTH II  $\beta$  (LH) immunoreactivity is found inside some vacuoles.

Fig 6 (B) An increase number of anti-GTH II  $\beta$  (LH) labeling gonadotrophs and the GTH II  $\beta$  (LH) immunoreactivity is found inside most vacuoles.

Fig 7 (B) A marked numerical increasing of anti- GTH II  $\beta$  (LH) labeling gonadotrophs in PPD and an increase of anti- GTH II  $\beta$  (LH) immunoreactivity in the cytoplasm of gonadotrophs. RPD, Rostral pars distalis; PPD, Proximal pars distalis; PI, Pars intermedia; PN, Pars nervosa.

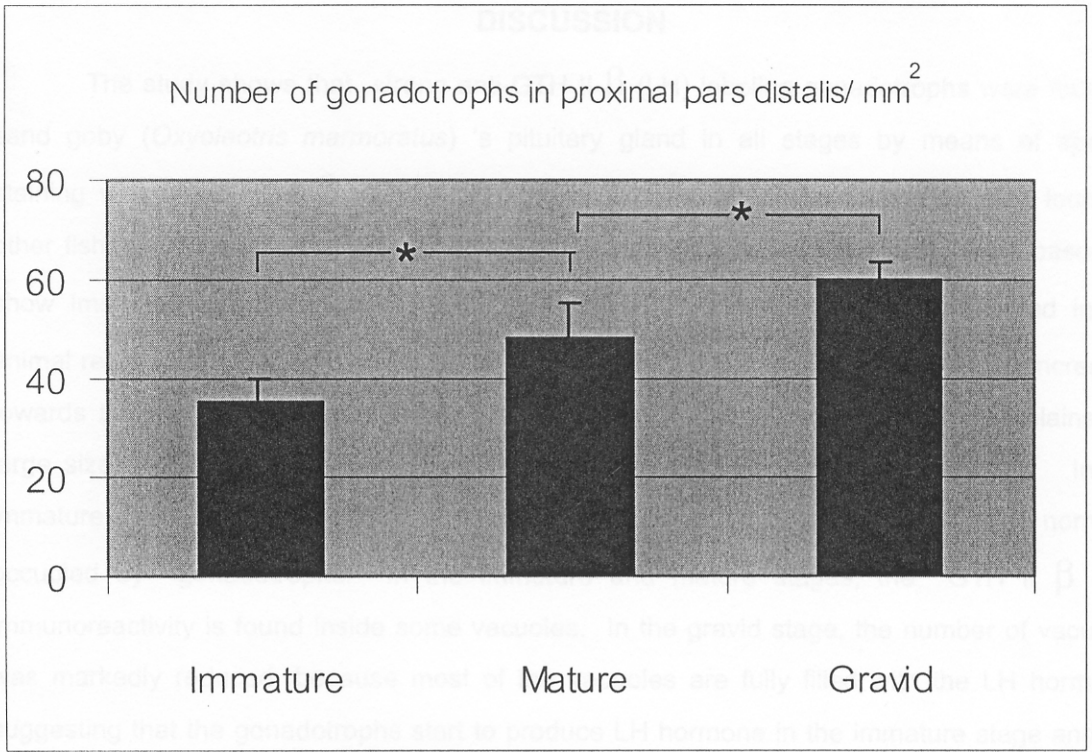


Fig. 8 The number of anti- GTH II  $\beta$  (LH) labeling gonadotrophs/mm<sup>2</sup> in PPD of the sand goby pituitary gland at different maturity stages of ovary. N= 6, one way ANOVA and Least-Significant Different LSD for post hoc analyses, \* = significant difference between two groups